CLAIMS

1. A method for producing a protected peptide fragment containing one or more modified amino acids or non-amino acids, which comprises preparing, on a weak acid-cleavable resin, a peptide fragment which has a desired sequence comprising amino acids or/and non-amino acids, at least one amino acid or non-amino acid of them being a modified amino acid or non-amino acid, represented by the formula 1; -A(R)- (wherein, A represents an amino acid or a non-amino acid, and R represents a substituent bound to a side chain of A which is introduced for modification), and in which one or more reactive functional groups which may cause an undesirable side reaction upon preparation of a peptide fragment, selected from the group consisting of a hydroxy group, an amino group, a guanidino group, an imidazolyl group, an indolyl group, a mercapto group and a carboxyl group, in a side chain of an amino acid or a non-amino acid, are protected with a protecting group, and

cleaving the peptide fragment from the weak acid-cleavable resin under weakly acidic conditions without elimination of the protecting group in the peptide fragment.

2. The method for producing a peptide fragment according to claim 1, which comprises (a) preparing, on a weak acid-cleavable resin, a peptide fragment having a desired sequence comprising amino acids or/and non-amino acids, in which one or more reactive functional groups which may cause an undesirable side reaction in preparation of a peptide fragment, selected from the group consisting of a hydroxy group, an amino

group, a guanidino group, an imidazolyl group, an indolyl group, a mercapto group and a carboxyl group, in a side chain of an amino acid or a non-amino acid, are protected with a protecting group, (b) deprotecting the protecting group without cleaving the peptide fragment from the weak acid-cleavable resin, when a protecting group is introduced in a reactive functional group in the side chain of an amino acid or a non-amino acid A which is to be modified with a substituent R, (c) modifying the deprotected side chain with a substituent R, and (d) cleaving the peptide fragment from the weak acid-cleavable resin under weakly acidic conditions without elimination of the protecting group in the peptide fragment.

3. The method for producing a peptide fragment according to claim 2, wherein a protecting group for a reactive functional group in a side chain of an amino acid or a non-amino acid A which is to be modified ~~by~~ with (Claim2の表記との整合) a substituent R is a silyl protecting group, and a quaternary ammonium fluoride is used for eliminating the protecting group.

4. The method for producing a peptide fragment according to claim 3, wherein the silyl protecting group is t-butyldimethylsilyl (TBDMS), t-butyldiphenylsilyl (TBDPS), triisopropylsilyl (TIPS), triisobutylsilyl (TIBS), t-hexyldimethylsilyl (ThxDMS) or triphenylsilyl (TPS), and the quaternary ammonium fluoride is tetrabutylammonium fluoride (TBAF), tetraethylammonium fluoride (TEF) or ammonium fluoride.

5. The method for producing a peptide fragment according

to any one of claims 1 to 4, wherein A is serine, threonine, cysteine, homocysteine, lysine, ornithine, glutamic acid, 2-aminoadipic acid, diaminoacetic acid, 2-aminomalonic acid, aspartic acid, tyrosine or asparagine, and R is bound to a reactive substituent in the side chain of A via an ester bond, an ether bond, a thioether bond, a disulfide bond, an amide bond, an O-glycoside bond or an N-glycoside bond.

6. The method for producing a peptide fragment according to claim 5, wherein A is serine or threonine, and R is bound to the hydroxy group in the side chain of A via an ester bond.

7. The method for producing a peptide fragment according to claim 6, wherein the peptide fragment is ghrelin or a derivative thereof, or a peptide fragment containing a modified amino acid in the ghrelin or a derivative thereof.

8. A method for producing a modified peptide or protein, which comprises (a) preparing a protected peptide fragment containing one or more modified amino acids or non-amino acids by the method described in any one of claims 1 to 7, (b) preparing a peptide fragment containing no modified amino acid or non-amino acid, and in which one or more reactive functional groups which may cause an undesirable side reaction, selected from the group consisting of a hydroxy group, an amino group, a guanidino group, an imidazolyl group, an indolyl group, a mercapto group and a carboxyl group, in the side chain of an amino acid or a non-amino acid, are protected, besides the peptide fragment of the (a), and condensing peptide fragments prepared in the (a) and the

(b).

9. The method for producing a modified peptide or protein according to claim 8, wherein condensation of the peptide fragments is performed by using a condensing agent.

10. The method for producing a modified peptide or protein according to claim 9, wherein the condensing agent is 2-(1-hydrobenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), 2-(1-hydrobenzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), diphenylphosphorylazide (DPPA), diphenylphosphorocyanidate (DEPC), diisopropylcarbodiimide (DIPC), dicyclohexylcarbodiimide (DCC) or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC).

11. The method for producing a modified peptide or protein according to claim 9, wherein the condensing agent is diisopropylcarbodiimide (DIPC), dicyclohexylcarbodiimide (DCC) or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), and condensation of the peptide fragments (a) and (b) using the condensing agent is performed in the presence of 1-hydroxybenzotriazole (HOBt), 1-hydroxysuccinimide (HOSu) or 3,4-dihydro-3-hydroxy-4-oxo-benzotriazine (HOOBt).

12. The method for producing a modified peptide or protein according to any one of claims 8 to 11, which comprises producing a protected peptide fragment containing no modified amino acid or non-amino acid, by an enzymatic method or/and a genetic

recombination method.

13. The method for producing a modified peptide or protein according to claim 12, wherein the protected peptide fragment containing no modified amino acid or non-amino acid is produced by a method comprising;

step (1); a step of culturing a cell transformed with an expression vector having one of a nucleotide sequence encoding a peptide having an amino acid sequence of the peptide fragment (hereinafter referred to as desired peptide, in the present claim 13) and a nucleotide sequence encoding a fusion protein optionally with a protective peptide added to the desired peptide via a linker sequence, and collecting the desired peptide or the fusion protein from the culture;

step (2); a step of cleaving and separating the protected peptide and, optionally, the linker sequence and the desired peptide from the resulting fusion protein, and optionally further purifying the desired peptide when the fusion protein is collected in the step (1); and

step (3); a step of protecting, with a protecting group, one or more reactive functional groups which may cause an undesirable side reaction, selected from the group consisting of a hydroxy group, an amino group, a guanidino group, an imidazolyl group, an indolyl group, a mercapto group and a carboxyl group, in the side chain of the desired peptide obtained in the step (1) or the step (2).

14. The method for producing a modified peptide or protein according to claim 13, wherein cleavage and separation of the

protective peptide and, optionally, the linker sequence and the desired peptide in the step (2) is performed at two steps using an OmpT protease or a derivative thereof and Kex2 protease or a derivative thereof.

15. The method for producing a modified peptide or protein according to claim 13 or 14, wherein the linker sequence is a sequence set forth in SEQ ID NO: 27.

16. The method for producing a modified peptide or protein according to any one of claims 12 to 15, wherein the peptide fragment is a peptide fragment containing no modified amino acid or non-amino acid in ghrelin or a derivative thereof.

17. The method for producing a modified peptide or protein according to any one of claims 12 to 16, wherein the protected peptide fragment containing no modified amino acid or non-amino acid is purified and stored in a solution having a pH of 4 to 8.

18. The method for producing a modified peptide or protein according to any one of claims 12 to 17, wherein the protecting group is a Boc group.

19. A method for producing a protected peptide fragment containing no modified amino acid or a non-amino acid, which comprises producing the peptide fragment by a method comprising:

step (1); a step of culturing a cell transformed with an expression vector having one of a nucleotide sequence encoding

a peptide having the desired amino acid sequence (hereinafter, referred to as desired peptide, in the present claim 19) and a nucleotide sequence encoding a fusion protein optionally with a protective peptide added to the desired peptide via a linker sequence, and collecting the desired peptide or the fusion protein from the culture;

step (2); a step of cleaving and separating the protective peptide and, optionally, the linker sequence and the desired peptide from the resulting fusion protein and, optionally further purifying this, when the fusion protein is collected in the step (1);

step (3); a step of protecting, with a protecting group, one or more reactive substituents which may cause an undesirable side reaction, selected from the group consisting of a hydroxy group, an amino group, a guanidino group, an imidazolyl group, an indolyl group, a mercapto group and a carboxyl group, in the side chain of the desired peptide obtained in the step (1) or (2); and

step (4); a step of purifying and storing the protected desired peptide obtained in the step (3) in a solution having a pH of 4 to 8.

20. The method for producing a protected peptide fragment containing no modified amino acid or non-amino acid according to claim 19, wherein the protecting group is a Boc group.

21. The method for producing a protected peptide fragment containing no modified amino acid or non-amino acid according to claim 19 or 20, wherein cleavage and separation of the

protective peptide and, optionally, the linker sequence and the desired peptide in the step (2) is performed at two steps using an OmpT protease or a derivative thereof and Kex2 protease or a derivative thereof.

22. The method for producing a protected peptide fragment containing no modified amino acid or non-amino acid according to any one of claims 19 to 21, wherein the linker sequence is a sequence set forth in SEQ ID NO: 27.

23. The method for producing a modified peptide or protein according to any one of claims 19 to 22, wherein the peptide fragment is a peptide fragment containing no modified amino acid or non-amino acid in ghrelin or a derivative thereof.